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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REPLY TO ATTN OF: Mr. Joseph M. Cummins, Biologist
EPA, Manchester Laboratory

DATE: September 19,1973

SUBJECT: Results of Oyster Embryo Bioassay of Duwamish River Bottom Sediments

TO: Mr. Arnold Gahler, Director
EPA, Region X Laboratory
Redmond, Washington

Attached for your information and review are the results of the oyster embryo bioassays conducted on selected Duwamish River bottom sediments. Also included is a brief description of the sampling method and assay procedure followed, as well as a few comments on the results.

Mr. John Sainsbury, EPA, Region X, was most interested in receiving this information. I would appreciate your providing him with a copy of this report after you have completed your review.

Joseph M. Cummins

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EFFECTS OF DUWAMISH RIVER BOTTOM SEDIMENTS ON PACIFIC OYSTER EMBRYOS

General Approach

Embryos of the Pacific oyster, Crassostrea gigas, were exposed for 48 hours to seawater suspensions of Duwamish River bottom sediments to measure the effect of the sediments on embryo survival and development.

Methods

(Sample Collection)

Bottom sediment cores were collected June 5, 1973 from several stations on the Duwamish River. The cores were taken with a gravity corer having a PVC tube liner. The liner was rinsed with river water between corings. Split samples of the core tops (sediment surface) and core bottoms (~3' depth) were placed in separate, chemically-clean, glass jars having aluminum foil-lined lids. The samples were then refrigerated. Later, the samples were independently subjected to various chemical analyses and bioassayed as described below.

(Sample Preparation)

Sediment samples were prepared for assaying in the following manner:

1. 100 g of well-mixed sediment was added to a 1-liter glass beaker and brought up to 500 ml with Burley Lagoon (Control) seawater (Salinity: 29.5 0/00).

2. This mixture was vigorously stirred for 2 minutes with a magnetic stirrer using a Teflon-coated stir bar. It was difficult, if not impossible, to obtain an entirely homogeneous mixture in this way because of the tendency of the heavier sediment particles to settle out.

3. Four decimal dilutions of the sediment suspensions were prepared in 1-liter, glass beakers by bringing appropriate volumes of the 500-ml "stock mixture" up to 1-liter with Burley Lagoon (Control) seawater. Duplicate sediment concentrations of 0.01, 0.1, 1, and 10 g/liter (wet wt.) were obtained in this manner.

4. These final sediment suspensions were well-mixed, then allowed to settle before being assayed. No additional aggitation was provided. Turbidity and pH determinations were made on the well-mixed preparations.

(Bioassay Procedure)

The sediments were assayed June 6, 1973 by the general oyster embryo technique of Woelke (1972). Efforts to induce spawning in female oysters were unsuccessful. Eggs were obtained, therefore, by removing portions of the tissue covering the gonad of a shucked oyster, placing the oyster in a beaker of seawater (~20 C), and allowing ripe eggs to fall freely into the surrounding water. When a sufficient number of eggs had been released, the oyster was removed and the eggs fertilized with about 3 ml of a stripped sperm suspension.

Each 1-liter test and control preparation was inoculated with 2.73×10^4 developing embryos. The cultures were covered with brown paper and incubated for 48 hours at approximately 20 C. The assay was terminated by carefully siphoning each culture through a 35 μm Nitex screen without disturbing the settled sediment. Samples of larvae collected in this fashion were quantitatively transferred to glass vials and preserved with 5% formalin. Normal and abnormal oyster larvae were enumerated under a microscope at 100X.

Results

The response of Pacific oyster embryos to seawater suspensions of Duwamish River bottom sediments is presented in Table 1.

Table 1. Response of Pacific oyster embryos to seawater suspensions of Duwamish River bottom sediments.

Sample Number	Sample & Type	Collection Date	Sediment Concentration (g/l) Wet Wt.	Sediment Concentration (g/l) Dry Wt.[a]	Turbidity FTUs[b]	pH	Oyster Embryo Response: Mean % Abnormal Larvae	Oyster Embryo Response: Relative % Survival
.....	Control Seawater[c]	6/6/73			1.4	8.0	0.91	100[e]
.....	Control Sediment[d]	6/6/73	0.01	-	1.3	8.0	1.29	101
			0.1	-	3.7	8.0	1.68	117
			1	-	60	8.0	5.73	110
			10	-	120	7.8	99.0	19.6
.....	Elliott Bay Seawater	6/~~21~~6/73						
	0'				1.4	7.9	2.39	118
	100'				0.5	7.9	2.16	101
	200'				0.8	7.9	1.70	102
23603	Sediment Core #2 Surface	6/5/73	0.01	0.0057	2.5	8.0	5.12	85.2
			0.1	0.05	12	8.0	3.18	89.1
			1	0.57	63	8.0	9.67	67.4
			10	5.7	160	7.9	6.97	62.6
23604	Sediment Core #2 ~3.5'	6/5/73	0.01	0.0048	2.7	8.0	1.49	87.8
			0.1	0.048	12	7.9	5.42	72.2
			1	0.48	105	8.0	11.9	43.9
			10	4.8	50	7.6	24.5	35.7

[a] Based on per cent dry wt. determinations performed by EPA, Redmond Laboratory

[b] Formazin Turbidity Units [c] Source: Burley Lagoon; Salinity: 29.5 0/00

[d] Source: Settling tank, Burley Lagoon [e] Assigned a survival value of 100%

Table 1. Continued

Sample Number & Type	Collection Date	Sediment Concentration (g/l) Wet Wt.	Sediment Concentration (g/l) Dry Wt.[a]	Turbidity FTUs[b]	pH	Oyster Embryo Response Mean % Abnormal Larvae	Oyster Embryo Response Relative % Survival
23607 Sediment Core #4 Surface	6/5/73	0.01	0.0055	1.8	8.0	4.21	82.6
		0.1	0.055	1.6	8.0	5.61	93.0
		1	0.55	52	8.0	11.7	44.8
		10	5.5	140	8.1	18.9	46.1
23608 Sediment Core #4 ~3.5'	6/5/73	0.01	0.0041	1.5	8.0	3.04	100
		0.1	0.041	1.8	8.0	3.21	81.3
		1	0.41	53	8.0	28.3	39.6
		10	4.1	140	7.9	26.1	36.1
23611 Sediment Core #6 Surface	6/5/73	0.01	0.0057	2.3	7.9	1.23	88.7
		0.1	0.057	7.5	8.0	1.98	77.0
		1	0.57	73	8.0	4.53	57.8
		10	5.7	90	7.9	8.77	49.6
23612 Sediment Core #6 ~3'	6/5/73	0.01	0.0056	2.8	7.9	0.62	96.5
		0.1	0.056	9.9	8.0	3.52	80.4
		1	0.56	85	7.9	12.7	46.1
		10	5.6	110	7.9	11.4	30.4
23625 Sediment Core #13 Surface	6/5/73	0.01	0.0017	1.6	7.9	2.14	81.3
		0.1	0.017	2.8	7.9	1.67	91.3
		1	0.17	17	7.9	0.28	78.3
		10	1.7	85	7.9	1.41	92.6

Table 1. Continued

Sample Number	Sample & Type	Collection Date	Sediment Concentration (g/l) Wet Wt.	Sediment Concentration (g/l) Dry Wt. [a]	Turbidity FTUs[b]	pH	Oyster Embryo Response Mean % Abnormal Larvae	Oyster Embryo Response Relative % Survival
23627	Sediment Core #15 Surface	6/5/73	0.01	0.0014	1.3	8.0	0.80	119
			0.1	0.014	5.6	7.9	0.64	102
			1	0.14	47	7.9	7.89	49.6
			10	1.4	65	7.9	35.9	28.7
23623	Sediment Core #16 Surface	6/5/73	0.01	0.0038	3.0	7.9	0.89	97.8
			0.1	0.038	13	8.0	6.21	38.7
			1	0.38	96	7.9	60.00	2.17
			10	3.8	96	7.9	-	0
23624	Sediment Core #16 ~3'	6/5/73	0.01	0.0061	3.0	7.9	0.60	108
			0.1	0.061	11	7.9	0.66	98.7
			1	0.61	95	7.9	13.3	42.6
			10	6.1	225	7.9	20.4	20.4

Comments

Oyster embryo responses reflected by larval abnormality rates greater than 5% exceeded the marine water quality criterion proposed by Woelke (1972) for waters used for fish or shellfish reproduction, rearing, or harvesting. The response of embryos to most Duwamish River sediments at concentrations equal to or greater than 1 g/liter (wet wt.) was in this category as well as being characterized by relatively low survival. Sediments from Core #13 (surface) were not toxic at any of the concentrations tested, while other sediments were toxic at concentrations less than 1 g/liter (wet wt.).

Care must be taken when considering the meaning of these data in terms of the actual aquatic environment. This fact is underscored by the response of oyster embryos to sediment collected from known shellfish growing waters, Burley Lagoon (Control Sediment). Under the static conditions of the bioassay, embryo response to concentrations of Burley Lagoon sediment equal to or greater than 1 g/liter (wet wt.) also exceeded Woelke's (1972) proposed water quality criterion.

Additional effort needs to be directed toward further study of the influence of low dissolved oxygen concentrations as well as high bacterial populations on the survival and development of oyster embryos under static bioassay conditions.

References

Woelke, C. E. 1972. Development of a receiving water quality criterion based on the 48-hour Pacific oyster (*Crassostrea gigas*) embryo. Washington State Department of Fisheries, Technical Report No. 9, 93 p.

Submitted by:

Joseph M. Cummins, Biologist
EPA, Manchester Laboratory
Manchester, Washington